Volume 223, number 1, 113–116 FEB 05204 October 1987

Cloning of human muscle phosphofructokinase cDNA

Hiromu Nakajima, Tamio Noguchi[+], Tomoyuki Yamasaki, Norio Kono, Takehiko Tanaka[+] and Seiichiro Tarui

Second Department of Internal Medicine, Osaka University Medical School, 1–1–50 Fukushima, Fukushima-ku, Osaka 553 and [+] *Department of Nutrition and Physiological Chemistry, Osaka University Medical School, 4–3–57 Nakanoshima, Kita-ku, Osaka 530, Japan*

Received 7 August 1987; revised version received 27 August 1987

Three overlapping cDNA clones for human muscle phosphofructokinase (HMPFK) covering the complete coding sequence were isolated. The sequence included a poly(A) tail, a 399 bp 3′-untranslated region, a 2337 bp coding region for 779 amino acid residues and a part of the 5′-untranslated region. Homologies between HMPFK and rabbit muscle phosphofructokinase (RMPFK) were 96% of the amino acids and 89% of the nucleotides in the coding region. Like RMPFK, HMPFK also possessed the internal homology between C- and N-halves in its primary structure. Cloning of HMPFK cDNA will help to identify the molecular defect in patients with glycogenosis type VII (HMPFK deficiency).

Phosphofructokinase; cDNA cloning; Glycogenosis; (Human muscle)

1. INTRODUCTION

Phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) is a key regulatory enzyme in the glycolytic pathway catalyzing the phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate. The enzyme defect in human muscle (glycogenosis type VII) was first reported by Tarui et al. [1]. To elucidate the molecular basis of the disease, the primary structure and corresponding nucleotide sequence of this enzyme should be clarified. Vora et al. [2] have recently reported a success in partial cDNA cloning of HMPFK, although the nucleotide sequence has not been described yet. Here, we report molecular cloning of HMPFK cDNA with complete coding sequence.

Part of this work has been presented by S. T. at the 84th Annual Scientific Session of the Japanese Society of Internal Medicine, April 1987, Tokyo, Japan

Correspondence address: H. Nakajima, Second Department of Internal Medicine, Osaka University Medical School, 1-1-50 Fukushima, Fukushima-ku, Osaka 553, Japan

Abbreviations: HMPFK (RMPFK): human (rabbit) muscle phosphofructokinase

The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession number Y00698

2. MATERIALS AND METHODS

A cDNA library of human muscle poly(A) RNA was prepared using the Okayama-Berg plasmid pCDV1 [3]. Only one clone, pHMP1.10 hybridized with a nick-translated RMPFK cDNA (a gift from Dr S.D. Putney and Dr P. Schimmel [4]). It contained 1.1 kb of insert with a poly(A) tail, a 399 bp 3′-untranslated region and a 528 bp C-terminal coding sequence. This clone was identified as a partial HMPFK clone since its coding nucleotide sequence retained 89% homology with the published RMPFK sequence [5].

Oligonucleotide (5′-AGT.GGT.ATA.GTT.CTC.ATT.GCA-3′) complementary to the se-

Published by Elsevier Publishers B.V. (Biomedical Division)
00145793/87/$3.50

quence downstream of the *Pst*I site of pHMP1.10 was used as a primer extension probe to construct the second library in λgt10 [6,7]. One of the clones, λHMPE4.1 hybridizing with *Pst*I/*Taq*I fragment of pHMP1.10 (HM-3 as shown in fig. 1) extended approximately 1.2 kb toward the 5'-region corresponding to 384 amino acid residues. *Ava*II/*Eco*RI fragment of λHMPE4.1 (HM-4B as shown in fig. 1) was digested with *Dde*I for the primer to construct the next library in λgt10. Clone λHMP2E1.8 hybridized with both HM-4B and RM-7 (5'-ATG.ACC.CAT.GAA.GAG.CAC. CAT-3', an oligonucleotide probe derived from the 5'-terminal coding sequence of RMPFK [5] including the initiation codon). It contained a 1.7 kb insert including a 930 bp coding sequence for 310 amino acids. Thus, three overlapping clones, pHMP1.10, λHMPE4.1 and λHMP2E1.8, covering complete coding sequence were obtained.

Oligonucleotides were made on an Applied Biosystems 380A synthesizer. Nucleotide sequence was determined using the dideoxy chain termination method [8]. Radiolabelled probes were prepared by nick-translation [9] or by random primer method [10] with [α-^{32}P]dCTP (3000 Ci/mmol) for cDNA probes and 5'-end labelling for oligonucleotide probes with [γ-^{32}P]ATP (6000 Ci/mmol) [9]. Other basic techniques were essentially the same as described [6,7,9,11].

Human muscle was obtained from a myopathy-free male patient with osteosarcoma when he underwent an operation. An apparently healthy portion of the resected muscle distal to the primary tumor site was used. Informed consent was obtained from the patient and the research was carried out in conformity with the declaration of Helsinki.

3. RESULTS AND DISCUSSION

A selected restriction map and the sequencing strategies for three clones are shown in fig. 1. As shown in fig. 2, HMPFK consisted of 779 amino acids with a molecular mass of 85 050 Da.

From Northern analysis of human muscle mRNA using the insert of pHMP1.10 as a probe, the size of HMPFK mRNA was estimated to be 3.15 kb (not shown), suggesting that the length of the poly(A) tail and 5'-untranslated region would sum up to about 400 bp. The size of the insert of

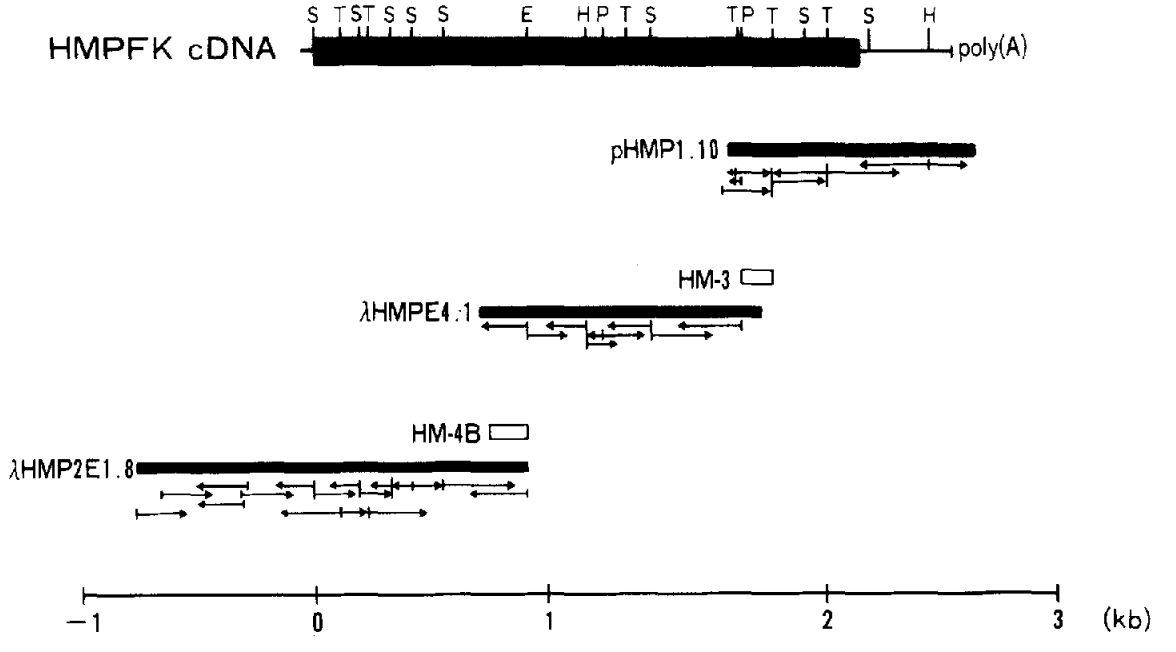

Fig. 1. Selected restriction map of HMPFK cDNA and sequencing strategies for the three overlapping clones (E, *Eco*RI; H, *Hin*dIII; P, *Pst*I; S, *Sau*3AI; T, *Taq*I).

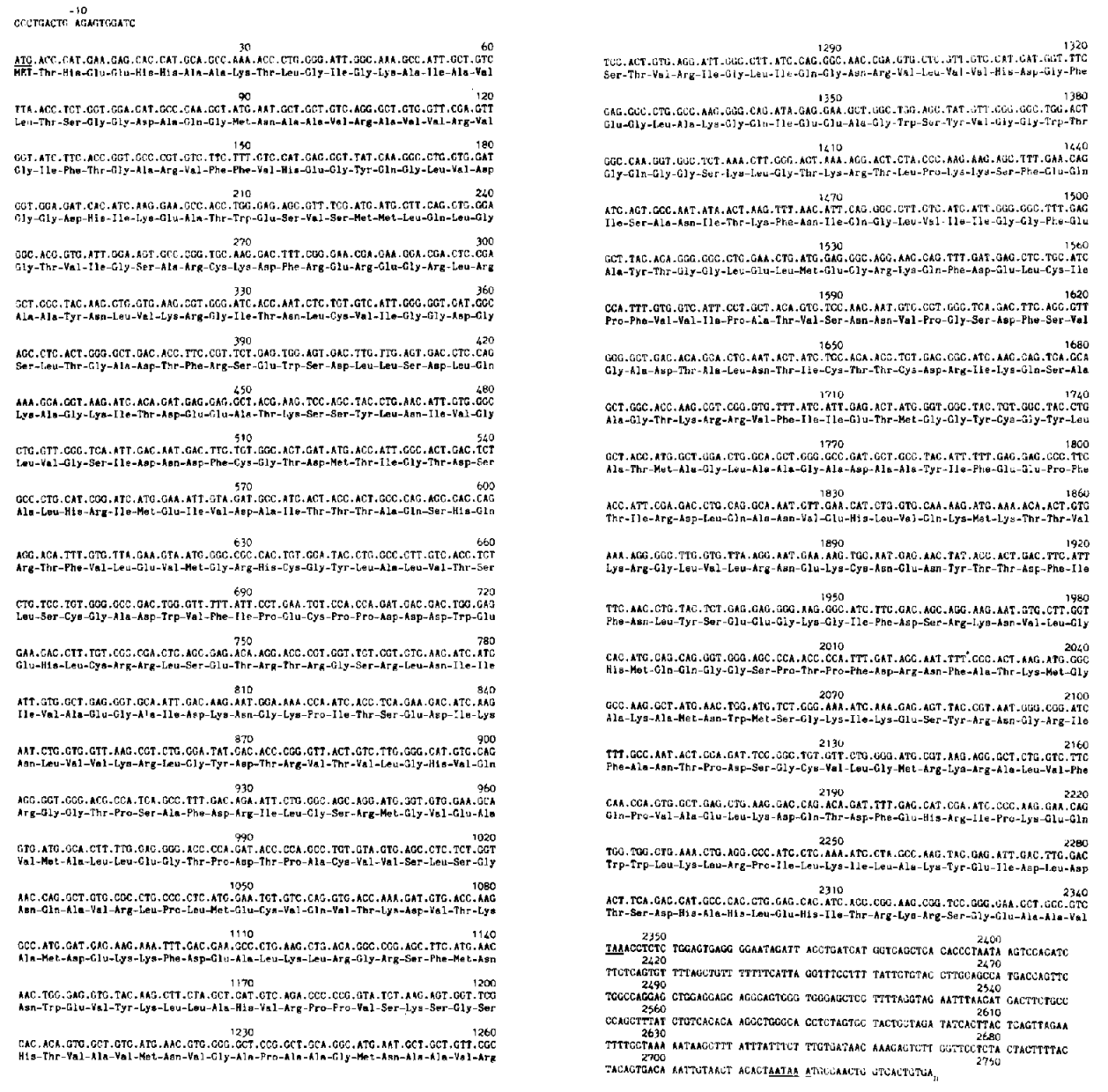

Fig. 2. Nucleotide sequence and deduced amino acid sequence of HMPFK. Putative initiation codon (ATG), termination codon (TAA) and polyadenylation signal (AATAAA) are underlined.

λHMP2E1.8 is longer than that expected from the result of the Northern analysis. This clone may have contained small unknown cDNA as a ligation artifact. Thus, the length of 5′-untranslated region needs further characterization. However, several lines of evidence indicate that the entire coding region of HMPFK is represented in fig. 2 and the ATG codon at position 1 is the initiation codon. First, the molecular mass of HMPFK has been estimated to be 85 kDa [12]. As described above, the molecular mass encoded by the sequence shown in fig. 2 is 85 050 Da. The total number of amino acid residues also matched that of RMPFK [5]. Second, a nonsense codon (TGA) was found at

position −12 followed by the open reading frame including a putative initiation codon (ATG) and a full-coding sequence comparable to RMPFK. Third, the initiation codon recognized by eukaryotic ribosomes possesses several consensus base compositions [13], of which ATG flanked by 80% adenine (A) at −3 position, as observed in this case, is one. This strongly suggests that ATG at position 1 is the initiation codon for HMPFK.

Homologies between HMPFK and RMPFK were very high both in nucleotides and amino acids (89% for 2337 bp coding sequence and 96% for 779 amino acid residues). In addition, the internal homology between N- and C-halves of its primary structure was also present in HMPFK as observed in RMPFK [5,14]. HMPFK differed from RMPFK by 29 amino acid residues, of which 20 changes occurred by single base substitution. Twenty mismatches appeared in the N-half, suggesting that the C-half sequence was preferentially conserved. It may be worth mentioning that a part of the C-half sequence is considered to contribute to the generation of a new effector site, fructose 1,6- and 2,6-bisphosphate binding site during the evolution of the enzyme by gene duplication [14].

The determined complete coding sequence for HMPFK will help in analysing not only the molecular defect of patients with glycogenosis type VII but also the regulatory mechanism of muscle glycolysis at the molecular level in the future biochemical investigations.

ACKNOWLEDGEMENTS

We thank Dr S.D. Putney of the Repligen Corp. and Dr P. Schimmel of Massachusetts Institute of Technology for kindly providing the RMPFK probe, Dr K. Hiroshima of the Department of Orthopedic Surgery, Osaka University Hospital for the help in obtaining human muscle. We also thank Dr M. Kawachi, Dr N. Hara, Mr K. Yamada and Dr M. Takenaka for preparing the manuscript. This work was supported in part by the Grants-in-Aids from the Ministry of Education, Science and Culture, Japan and the Basic Research Grants from MDA of the Unites States.

REFERENCES

[1] Tarui, S., Okuno, G., Ikura, Y., Tanaka, T., Suda, M. and Nishikawa, M. (1965) Biochem. Biophys. Res. Commun. 19, 517–523.
[2] Vora, S., Hong, F. and Olender, E. (1986) Biochem. Biophys. Res. Commun. 135, 612–615.
[3] Okayama, H. and Berg, P. (1983) Mol. Cell. Biol. 3, 280–289.
[4] Putney, S.D., Herlihy, W.C. and Schimmel, P. (1983) Nature 302, 718–721.
[5] Lee, C.P., Kao, M.C., French, B.A., Putney, S.D. and Chang, S.H. (1987) J. Biol. Chem. 262, 4195–4199.
[6] Hames, B.D. and Higgins, S.J. (1985) Nucleic Acid Hybridisation. A Practical Approach, IRL, Oxford.
[7] Glover, D.M. (1985) DNA Cloning, vol. 1, A Practical Approach, IRL, Oxford.
[8] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.
[9] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
[10] Feinberg, A.P. and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13.
[11] Noguchi, T., Inoue, H., Chen, H.L., Matsubara, K. and Tanaka, T. (1983) J. Biol. Chem. 258, 15220–15223.
[12] Karadsheh, N.S., Uyeda, K. and Oliver, R.M. (1977) J. Biol. Chem. 252, 3515–3524.
[13] Kozak, M. (1984) Nature 308, 241–246.
[14] Poorman, R.A., Randolph, A., Kemp, R.G. and Heinrikson, R.L. (1984) Nature 309, 467–469.